Title

Clocks for the city: circadian differences between forest and city songbirds

Author information

Dominoni, D. M.[1,2,3*], Helm, B.[1,2,3], Lehmann, M.[2], Dowse, H. B.[4], Partecke, J.[1,2]

Affiliations

[1]Department of Migration and Immuno-ecology, Max Planck Institute for Ornithology, Radolfzell 78479, Germany

[2]Department of Biology, University of Konstanz, Konstanz 78457, Germany

[3]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, G12 8QQ, UK

[4]School of Biology and Ecology, University of Maine, Orono, ME 04469, USA

* Corresponding author

E-mail: ddominoni@orn.mpg.de

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Abstract

To keep pace with progressing urbanization organisms must cope with extensive habitat change. Anthropogenic light and noise have modified differences between day and night and may thereby interfere with circadian clocks. Urbanized species, like birds, are known to advance their activity to early morning and night hours. We hypothesized that such modified activity patterns are reflected by properties of the endogenous circadian clock. Using automatic radio-telemetry we tested this idea by comparing activity patterns of free-living forest and city European blackbirds (*Turdus merula*). We then recaptured the same individuals and recorded their activity under constant conditions. City birds started their activity earlier and had faster but less robust circadian oscillation of locomotor activity than forest conspecifics. Circadian period length predicted start of activity in the field and this relationship was mainly explained by fast-paced and early-rising city birds. Although based on only two populations, our findings point to links between city-life, chronotype and circadian phenotype in songbirds and potentially in other organisms that colonize urban habitats, and highlight that urban environment can significantly modify biologically important rhythms in wild organisms.

1. INTRODUCTION

We live in the urban millennium, when global change and rapid expansion of cities hasten the loss of animal and plant species [1]. Yet despite dramatic alteration of the environment by urban sprawl, many wild organisms succeeded to colonize man-made habitats. Their surprising ability to thrive under novel environmental conditions has sparked increasing biological interest in urbanization [2]. The vast majority of studies on effects of urbanization on wild species has focused on the response to spatial habitat change, but urbanization also modifies temporal niches. For example, artificial light at night has been suggested to alter temporal activity patterns and physiology in different species among reptiles, birds and mammals [3–6]. Moreover, noise in urban areas has been suggested to promote nocturnal activity in diurnal species, at least in songbirds [7].

Daily cycles of behavior and body function are ubiquitous phenomena and are thought to be evolutionary adaptations to the earth's 24-h rotation [8]. The daily alternation of activity and rest is not a mere response but an entrainment of the endogenous circadian clock to cyclic environmental signals. Organisms benefit from the synchronization of the circadian clock with environmental cycles, mainly the light-dark cycle, for maximizing their fitness [9]. In humans, the shift to the 24-h society typical of urban areas in developed countries can disrupt synchronization between circadian physiological functions and daily activities like sleep and food intake, with serious health implications [10]. Such a change in human lifestyle affects also other species through shared urban habitats, but it is currently unclear whether endogenous circadian clocks are implicated in responses to urbanization.

To address temporal implications of city life we compared common songbirds, European blackbirds (*Turdus merula*), from a city and forest population. Blackbirds have been long associated with humans in Europe, and are an efficient model species because they thrive in

cities where they display nocturnal behavior, especially during early morning hours [11]. Our main objective was to examine variation in circadian traits between the two populations, and to relate this variation, if present, to variation in chronotype in the wild. Chronotype is defined as the timing of an individual relative to an external synchronizing cue (*Zeitgeber*), and results from interactions between the endogenous circadian clock and environmental factors [12]. Assignment of a particular chronotype to an individual requires within-individual consistency in timing. Across a population, variation in chronotype correlates with circadian properties in many species including humans [13]. Early chronotypes typically have shorter circadian period length (tau, τ) than late chronotypes [14]. In view of the nightly and early morning activities exhibited by European blackbirds, we hypothesized that individuals from urban areas had faster free-running circadian clocks than their forest conspecifics. We furthermore proposed that in urban habitats, where *Zeitgeber* information is compromised by anthropogenic light and noise, organisms may fare better with relatively labile circadian clocks.

2. MATERIAL AND METHODS

(a) Activity recording of free-living animals

During the breeding season of 2010, we captured 12 adult male European blackbirds (forest, N = 6; city, N = 6) in various locations in the city of Munich (Germany, 48º 07´ N, 11º 34´ E; 518 m asl) and in a rural forest near the village of Raisting (47º 53´ N, 11º 04´ E, 553 m asl), 40 km south-west of Munich (see electronic supplementary material, figure S1). Birds were equipped with 2.2 grams pulse radio-transmitters (Sparrow Systems, IL, USA) and immediately released at their capture site. Daily activity was recorded using Automated Recording Units (ARUs, Sparrow Systems, IL, USA). The ARU was placed close to the territory of a bird and connected to an H-antenna (Sparrow Systems, IL, USA). The unit was programmed to scan every minute

for the corresponding frequency of each bird and to record the signal strength of the radio-transmitter pulse. We used the data from birds which spent at least a week within the range of the antennas and thus provided continuous and high quality recordings (mean ± s.d. = 21 ± 8 days, N = 6 forest birds and 6 city birds). We used the change in signal strength over time to infer the time of start of activity, as previously described [15]. Briefly, the time of start and end of activity was estimated to the minute by comparing consecutive data points and scoring the minute when a change in signal strength was greater than 4 db. We used start of activity as a proxy for chronotype because of evidence that the morning hours are of particular ecological and evolutionary relevance for songbirds [4,16–18], but we also examined end of activity and total duration of daily activity, and related timing to day-length information from astronomical charts. For every day of recording, we standardized start, end and duration of activity by subtracting each value from the start of morning civil twilight, end of evening civil twilight and duration of daylight for Raisting and Munich, as available at the United States Navel Observatory database (www.usno.navy.mil/USNO).

(b) Recording of circadian rhythms in captivity

Tagged birds were recaptured within the same breeding season between the end of April and end of June 2010 and were transported in cloth cages to our facilities in Andechs, Germany (47º 58´ N, 11º 11´ E, 690 m asl). In addition, we captured additional adult male birds for which activity was not recorded in the wild (forest = 8, city = 8). In total we captured 14 birds from each population. Birds were placed in individual cages (width x height x length: 45x70x80 cm), each of them located in a light-proofed, sound-insulated chamber. Once transported to our facilities, birds were immediately placed under constant dim light (LL_{dim}, 0.3 lux, Tungsten warm bulb, Osram, Germany). Food (mealworms and mixed dry insects, Aleckwa, Germany) was provided

121 at random times during day and night to prevent birds from synchronizing to feeding cycles.
122 Drinking water was available ad libitum. Activity was recorded in LL_{dim} for an average of 10
123 days per bird (10 ± 2 days). Afterwards, birds were put under light/dark cycles (LD, 500 lux,
124 cool white fluorescent bulb, Osram, Germany) which followed the natural seasonal variation of
125 photoperiod in Andechs. Locomotor activity was recorded over the entire duration of the
126 experiment through a passive infrared sensor mounted on each cage (Intellisense, CK Systems,
127 Eindhoven, The Netherlands). Movements were counted and stored as two minutes bins into a
128 computer. All the experimental procedures were carried out in accordance with the guidelines of
129 the relevant German agencies.
130
131 ***(c) Analysis of circadian traits***
132 We employed methods of digital signal analysis to extract relevant circadian information from
133 the activity recorded in constant dim light conditions. These techniques are reliable and have
134 been used extensively [19,20]. To run the analysis, we used the libraries written by Harold
135 Dowse and described in [21]. We removed the first day of activity recording in all analyses to
136 limit possible bias in the data due to birds habituating to the new environment.
137 First, we used autocorrelation analysis to assess the strength of the periodicity in LL_{dim} as
138 described by Levine *et al*. [19]. Briefly, we calculate the coefficient of temporal autocorrelation
139 at each time lag and plotted it over time. We then assessed the strength of the rhythm as the
140 height of the third peak in the autocorrelation plot, a measure that has been named Rhythmicity
141 Index [19]. The third peak was taken because considered the most reliable point were to measure
142 the strength of the rhythmicity [19]. To assess whether a bird was rhythmic at all, we calculated
143 the 95 % acceptance region for the null hypothesis of no correlation. If the third peak in the
144 autocorrelation plot exceeded the acceptance region we defined this bird as rhythmic and used its

data in maximum entropy spectral analysis (MESA) to estimate the circadian period length. MESA works by fitting an autoregressive model to the time-series in order to compute a spectrum. We referred to [22] for details and validation tests of this technique. Graphic examples for two representative city and forest birds are shown in the electronic supplementary material, figure S4. After release from LL_{dim} into LD, the birds showed an unexpected pattern of daily activity: most birds shifted their active phase into the dark period (see electronic supplementary material, figure S5). This behaviour is hard to interpret and could, for example, represent nocturnal restlessness that typically occurs during migration or following captivity [23]. Because the biological interpretation would have been unclear, we did not include this aspect of the data into analyses.

(d) Statistical analysis

All statistical tests were computed with software R 2.13.0 [24]. We applied a significance level of $\alpha = 0.05$.

To test for differences in the start, end and duration of daily activity between forest and city birds we used linear mixed models (LMMs) from the R package *nlme* [25]. In all models we included origin and τ as fixed factors and date as covariate. We modeled random intercept dependent on individual to correct for repeated measures. Heteroscedasticity was present in all models since variance in the residuals was higher in city than forest birds. We corrected for this including weights on the residuals by origin. We removed one outlier from the urban population in the analysis of end of activity, because its mean exceeded of two standard deviations the mean of all other individuals pooled together. Within-individual variation and between individual variation in start, end and duration of daily activity in the field were extracted from the model outputs and used to calculate repeatability (i.e. the proportion of phenotypic variance explained by individual

169 [26]) following Lessells and Boag [27]. The difference between city and forest birds in the
170 between-individual variation in start of daily activity was assessed by Levene's test from the R
171 package *lawstat* (http://cran.r-project.org/package=lawstat).
172 Circadian traits were tested by independent two-sample test. We used a t-test to compare the
173 Rhythmicity Index between forest and city birds. To compare τ in the two populations we
174 switched to a non-parametric Mann-Whitney test because assumptions of normality were not
175 met. Furthermore, since the photoperiod experienced by an animals prior to be recorded under
176 constant conditions can affect its circadian period length ([28]), we tested for this potential effect
177 with a linear model (LM) including τ as response variable and date of recapture from the field
178 (activity recording under LL_{dim} started on the same day) as explanatory variable.
179

180 **3. RESULTS**

181 City birds started their activity on average 29 ± 17 minutes (mean ± s.d.) before civil twilight,
182 while forest birds differed by synchronizing activity to the onset of twilight (mean onset ± s.d. =
183 0 ± 3 minutes, linear mixed model, df = 9, P = 0.0093, figure 1 and table 1). Between-individual
184 variation in start of activity was higher in city than in forest blackbirds (Levene's test: test
185 statistic = 7.44, P < 0.001, table S1). Within-individual variation was also higher in city than in
186 forest individuals (variance forest = 80.2, variance city = 351.04; table S1). Since the difference
187 between city and forest birds in both between and within-individual variation was very similar,
188 repeatability estimates for the two populations were very close (forest R = 0.51, city R = 0.50;
189 table S1). At the end of the day, city birds extended their activity on average 6 ± 9 minutes later
190 into the evening than forest birds, although the difference was not significant (mean ± s.d., linear
191 mixed model, df = 9, P = 0.52, figure S2 and table 1). Therefore city birds were active for longer
192 than forest birds, on daily average by 40 ± 14 minutes per day (mean ± s.d., linear mixed model,

df = 9, P = 0.0043, figure S3 and table 1). All investigated aspects of timing in the wild showed high repeatability (table S1), indicating that activity patterns were consistent properties of individuals within the study.

City birds showed clearly reduced circadian rhythmicity compared to forest birds, as measured by the rhythmicity index (t-test: T = 2.08, df = 25.67, P = 0.048, figure 2a), supporting the idea of more labile circadian clocks in the city. For analyses of period length, the birds that were found to be arrhythmic (N = 0 for forest and N = 5 for city) were excluded because periodicity could not be determined. In the remaining city birds circadian period length was on average shorter by 50 minutes than in forest birds (mean ± s.d., forest birds = 23 h 45 min ± 37 min, city birds = 22 h 55 min ± 35 min; Mann-Whitney test: U = 26, P = 0.021, figure 2b). The start date of recording under LL_{dim} (as a proxy for the daylength experienced by the birds prior to constant conditions) did not affect τ (LM: t = 0.66, P = 0.52, Fig. S5).

We found a positive linear relationship between τ and onset of activity. Indeed, fast-paced individuals with shorter free-running periodicities were the birds waking up earlier in the wild (LMM, df = 9, P = 0.0027, figure 3 and table 1). However, this relationship was absent in birds with slower clocks, in which start of activity was closely linked to onset of civil twilight. Thus, because blackbirds from urban and rural sites differed in period length, the relationship between τ and chronotype was influenced by site (LMM, df = 9, P = 0.009, figure 3 and table 1). In the fast-paced city birds, early-rising was related to the pace of the circadian clock, whereas forest birds showed variation in τ but consistently synchronized their morning activity to civil twilight (figure 1, 3, 4). In contrast, neither end nor total duration of daily activity were related to τ (LMMs: End of activity: df = 9, P = 0.81; Duration of activity: df = 9, P = 0.69, table 1). In all these models date was included as covariate, so that we considered effects of τ and origin to be independent of date.

4. DISCUSSION

The causes and consequences of variation in circadian period length between natural populations of the same species are crucial for the understanding of how organisms adapt to changes in their temporal environment [29,30]. Here we show that two populations of city and forest European blackbirds differ in both chronotype and circadian traits. The shift towards shorter circadian period length in the urban population is mirrored by behavior in the wild, because city birds started their activity earlier than forest birds. Furthermore, chronotype of city birds was correlated with the endogenous periodicity of their circadian clock under constant conditions, whereas the timing of onset and end of daily activity in forest birds was more closely related to civil twilight. Overall we conclude that urbanization may modify not only daily organization of activities, but may also alter endogenous circadian rhythmicity and its interaction with the *Zeitgeber* in wild animals. However, our conclusion is based on only one forest and one city population. Therefore in order to consolidate an effect of urbanization on circadian timing, as distinguished from other processes, data from additional rural and urban sites will be necessary.

Given our current data, we can merely speculate about the origin of these observed differences in chronotype and circadian traits between city and forest blackbirds. The causes could be environmentally-induced and/or intrinsic. For instance, city and forest habitats differ in several environmental characteristics that could influence temporal patterns of activity, such as daily noise patterns and artificial light at night. In particular, recent work, including our own studies on seasonal physiology of urban and forest blackbirds [6], suggested that the detection of daylength might be altered by the artificial illumination at night [4,6]. In our study city birds could have perceived the “lights on” signal earlier than forest birds, which may have advanced chronotype (i.e. onset of activity in the field)[6] and promoted after-effects on τ [28]. 24 h light profiles for

our two study sites are shown in fig. S6. Our measurements suggest that patterns of light intensity differed between the urban and rural habitat, but more detailed data on the individual exposure to light would be necessary to directly link light-dark cycles to chronotype and circadian rhythms. If altered daylength perception seems a plausible reason to explain the difference in chronotypes between the two populations, circadian period lengths were surprisingly robust with respect to after-effects of prior photoperiodic condition, since τ was independent of date (Fig. S5). Another process known to affect both the duration of daily activity and τ is seasonal change in reproductive physiology [31,32]. In order to minimize this potential confounding effect, we sampled blackbirds during May and June when both study populations exhibit their peak breeding stage during the reproductive season [33].

A fascinating hypothesis is that these observed differences are the result of micro-evolutionary changes to the new life in cities of this originally forest species. Possible evolutionary implications of a link between circadian traits and chronotypes are suggested by two recent studies and by general evidence. For songbirds performance during early morning hours is particularly important and may promote selection for early start of activity. Early birds increased reproductive success by greater extra-pair copulation opportunities in several studies [4,16] and were more efficient at territorial defense, e.g., by early dawn song [17,18]. Kempenaers et al. have shown that male blue tits (*Cyanistes caeruleus*) which occupied territories near streetlamps sang earlier in the morning, even before civil twilight. These males had also the highest rate of extra-pair paternity in the population, suggesting that early awakening as a response to artificial light at night could confer fitness benefits [4]. Hence one potential scenario may be conceivable: colonization of urban areas may increase selection for early chronotypes, which in turn would ease the exploitation of new temporal niches into the night. To the extent that chronotype depends on circadian properties, selection should also affect the circadian clock. A further recent

study pointed directly to reproductive benefits of fast circadian clocks. In wild-derived great tits (*Parus major*), chicks from extra-pair fathers had shorter circadian period length than the within-pair offspring. The same study also showed that circadian period length was highly heritable [34]. High heritability of a trait is one of the prerequisite for natural selection to act on this trait. Thus, assuming similar heritability of timing in blackbirds, selection in favor of early chronotypes in urban environment could lead to micro-evolutionary adjustments of both chronotype and underlying circadian traits.

Although the hypothesis of natural selection favouring faster clocks and early risers in urban habitats seems conceivable and exciting, results from our wild-caught subjects do not allow distinguishing between genetic difference, developmental plasticity or previous exposure to a particular environment. These three mechanisms are not mutually exclusive. All three processes are known to have the potential to alter circadian rhythmicity. For instance, Tauber et al. have shown that a recent mutation in the circadian clock gene *timeless* has altered diapause in *Drosophila melanogaster* [35]. In addition, epigenetic and specifically maternal effects can alter endogenous rhythmicity. For example, in quails the circadian phenotype of the mothers can predict that of their young [36]. Finally, as already mentioned above, after-effects of previous environmental exposure may affect circadian period length τ [28], for example depending on the amplitude and length of the photoperiod an animal experiences [37–40]. For the future we suggest possible experiments in order to better understand the mechanism behind the observed differences between city and forest-dwelling animals. We need experiments in which we record τ not only in wild-caught, but also in hand-reared forest and city birds. If differences in τ will not be found in hand-reared animals, this would strongly hint against genetic difference between forest and city populations, and either developmental plasticity or environmental effect might be considered. In order to test for developmental effects, eggs could be cross-fostered between the

two populations and subsequently τ could be recorded in nestlings raised from either their natural mother or a female in the opposite environment. Finally, to test for after-effects of artificial light at night, we would suggest to record τ after exposing forest and city birds to the same photoperiod. Effects of additional light at night could then be tested by separate treatments of both groups.

The distribution of chronotypes in our two populations closely fits theoretical predictions by Roenneberg et al. [12] based on oscillatory theory. The authors used empirical data of circadian periodicity in human beings for modeling possible chronotype distributions in relation to *Zeitgeber* strength. They suggested that with increasing *Zeitgeber* strength of light, chronotypes should be less variable and more closely synchronized, implicating that chronotype distribution should depend on the *Zeitgeber* conditions a population experiences (figure 4, panel a). The distributions of chronotypes in city and forest blackbirds are in line with this theory (figure 4, panel b). Birds living in the forest habitat showed a distribution with a sharp and high peak and low variance around the mean. Conversely, the chronotype of city birds showed a lower peak and higher between-individual variation than that of forest birds, mirroring the distribution of humans subjected to weak *Zeitgebers*. In addition, city birds were less consistent in their timing of morning activity than forest birds. Analysis of underlying rhythms suggests two circadian features that may affect activity patterns in the city, one being a possibly closer link of chronotype to period length in city birds than in their rural counterparts, as discussed above. The second feature is the higher heterogeneity in timing of onset of morning activity is mirrored by the weakness of the circadian rhythmicity under constant laboratory conditions. City birds were clearly less rhythmic than forest conspecifics as suggested by the index of robustness. Interestingly, the five city birds that were found to be arrhythmic were all sampled at the same downtown location in the central business district of Munich, whereas the remaining city birds

313 originated from other urban locations including parks, cemeteries and botanical gardens.
314 Although anecdotal, this observation supports the idea that loss of circadian rhythmicity is
315 related to inhabiting highly urbanized areas where environmental time cues may be less precise.
316 We propose that urban environments could be seen as habitats where the time information is
317 noisy and weak, thereby giving greater room for alternative temporal activity patterns if a
318 potential advantage is present.
319 The consequences of urbanization on daily and seasonal organization and well-being are
320 currently raising strong interest in both scientists and general public [8,41], especially in respect
321 to light at night and circadian biology [42,43]. Our study is novel in showing that changed
322 activity patterns are associated with altered circadian rhythmicity not just in humans [12,44] but
323 also in wild animals thriving in urban areas. We compared city and forest European blackbirds,
324 but we suggest that our framework and findings may be relevant for scientists looking for a link
325 between circadian rhythms, environmental change and daily activity in other species (for
326 example see [3]). One fascinating explanation for the observed differences in circadian traits
327 between forest and city birds could be selection for early chronotypes in urban habitats, possibly
328 due to potential reproductive advantages for early birds. The environmental pressures which
329 could have promoted the shift in both endogenous and overt rhythmicity remain to be elucidated,
330 but we suggest light pollution as a potential candidate. We believe that given the fitness and
331 health implications of circadian disruption [45], there is urgent need for scientists to understand
332 the costs and benefits of altered circadian rhythmicity in urban areas.
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REFERENCES

1 Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X. & Briggs, J. M. 2008 Global change and the ecology of cities. *Science* **319**, 756–760.

2 Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E. & Hope, D. 2006 From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol. Evol.* **21**, 186–191.

3 Longcore, T. & Rich, C. 2006 *Ecological consequences of artificial night lighting*. Washington, DC: Island Press.

4 Kempenaers, B., Borgström, P., Loës, P., Schlicht, E. & Valcu, M. 2010 Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Current Biology* **20**, 1735–1739.

5 Rotics, S., Dayan, T. & Kronfeld-Schor, N. 2011 Effect of artificial night lighting on temporally partitioned spiny mice. *Journal of Mammology* **92**, 159–168.

6 Dominoni, D., Quetting, M. & Partecke, J. 2013 Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society B: Biological Sciences* **280**. (doi:10.1098/rspb.2012.3017)

7 Fuller, R. A., Warren, P. H. & Gaston, K. J. 2007 Daytime noise predicts nocturnal singing in urban robins. *Biology Letters* **3**, 368–370.

8 Foster, R. G. & Kreitzmann, L. 2004 *Rhythms of life: The biological clocks that control the daily lives of every living thing*. New Haven, CT: Yale University Press.

9 Dunlap, J. C., Loros, J. J. & DeCoursey, P. J. 2004 *Chronobiology. Biological timekeeping*. Sunderland, Massachusettes: Sinauer Associates.

10 Klerman, E. B. 2005 Clinical aspects of human circadian rhythms. *J. Biol. Rhythms* **20**, 375–86.

11 Nemeth, E. & Brumm, H. 2009 Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanization? *Animal Behaviour* **78**, 637–641.

12 Roenneberg, T., Wirz-Justice, A. & Merrow, M. 2003 Life between clocks: daily temporal patterns of human chronotypes. *J. Biol. Rhythms* **18**, 80–90.

13 Kyriacou, C. P., Peixoto, A. A., Sandrelli, F., Costa, R. & Tauber, E. 2008 Clines in clock genes: fine-tuning circadian rhythms to the environment. *Trends Genet.* **24**, 124–132.

14 Brown, S. A., Kunz, D., Dumas, A., Westermark, P. O., Vanselow, K., Tilmann-Wahnschaffe, A., Herzel, H. & Kramer, A. 2008 Molecular insights into human daily behavior. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 1602–1607.

15 Bisson, I. A., Butler, L. K., Hayden, T. J., Romero, L. M. & Wikelski, M. C. 2009 No energetic cost of anthropogenic disturbance in a songbird. *Proceedings of the Royal Society B: Biological Sciences* **276**, 961–969.

16 Poesel, A., Kunc, H. P., Foerster, K., Johnsen, A. & Kempenaers, B. 2006 Early birds are sexy: male age, dawn song and extrapair paternity in blue tits, Cyanistes (formerly Parus) caeruleus. *Animal Behaviour* **72**, 531–538.

17 Amrhein, V. & Erne, N. 2006 Dawn singing reflects past territorial challenges in the winter wren. *Animal Behaviour* **71**, 1075–1080.

18 Kacelnik, A. & Krebs, J. R. 1983 The dawn chorus in the great tit (Parus major): proximate and ultimate causes. *Behaviour* **3**, 287–308.

19 Levine, J. D., Funes, P., Dowse, H. B. & Hall, J. C. 2002 Signal analysis of behavioral and molecular cycles. *BMC Neurosci.* **3**.

20 Krishnan, B., Levine, J. D., Lynch, M. K., Dowse, H. B., Funes, P., Hall, J. C., Hardin, P. E. & Dryer, S. E. 2001 A new role for cryptochrome in a Drosophila circadian oscillator. *Nature* **411**, 313–317.

21 Dowse, H. B. 2007 Statistical analysis of biological rhythm data. In *Circadian rhythms: methods and protocols (Methods in Molecular Biology, vol. 362)* (ed E. Rosato), pp. 29–45. Totowa, New Jersey: Humana Press.

22 Dowse, H. B. & Ringo, J. M. 1991 Comparisons between "periodograms" and spectral analysis: apples are apples after all. *J. Theor. Biol.* **148**, 139–144.

23 Mukhin, A., Grinkevich, V. & Helm, B. 2009 Under cover of darkness: nocturnal life of diurnal birds. *J. Biol. Rhythms* **24**, 225–231. (doi:10.1177/0748730409335349)

24 2011 *R Development Core Team. R: A language and environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

25 Pinheiro, J. C. & Bates, D. M. 2000 *Mixed-effect models in S and S-PLUS*. New York: Springer Verlag.

26 Falconer, D. S. & Mackay, T. F. C. 1996 *Introduction to quantitative genetics*. New York: Longman.

27 Lessells, C. & Boag, P. 1987 Unrepeatable repeatabilities: a common mistake. *The Auk* **104**, 116–121.

28 Pittendrigh, C. 1960 Circadian rhythms and the circadian organization of living systems. *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159–184.

29 Sawyer, L. A., Hennessy, J. M., Peixoto, A. A., Rosato, E., Parkinson, H., Costa, R. & Kyriacou, C. P. 1997 Natural variation in a Drosophila clock gene and temperature compensation. *Science* **278**, 2117–2120.

30 Michael, T. P., Salomé, P. A., Yu, H. J., Spencer, T. R., Sharp, E. L., McPeek, M. A., Alonso, J. M., Ecker, J. R. & McClung, C. R. 2003 Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* **302**, 1049–1053.

31 Gwinner, E. 1967 Circannuale Periodik der Mauser und der Zugunruhe bei einem Vogel. *Naturwissenschaften* , 1967–1967.

32 Gwinner, E. 1974 Testosterone induces splitting of circadian locomotor activity in birds. *Science* **185**, 72–74.

33 Partecke, J., Van't Hof, T. J. & Gwinner, E. 2005 Underlying physiological control of reproduction in urban and forest-dwelling European blackbirds Turdus merula. *Journal of Avian Biology* **36**, 295–305.

34 Helm, B. & Visser, M. E. 2010 Heritable circadian period length in a wild bird population. *Proc. R. Soc. London B* **277**, 3335–3343.

35 Tauber, E. et al. 2007 Natural selection favors a newly derived timeless allele in Drosophila melanogaster. *Science* **316**, 1895–1898.

36 Formanek, L., Richard-Yris, M.-A., Houdelier, C. & Lumineau, S. 2009 Epigenetic maternal effects on endogenous rhythms in precocial birds. *Chronobiology International* **26**, 396–414. (doi:10.1080/07420520902892433)

37 Pittendrigh, C. & Daan, S. 1976 A functional analysis of circadian pacemakers in nocturnal rodents. I. The stability and lability of spontaneous frequency. *J. Comp. Physiol. A* **252**, 223–252.

38 Aschoff, J. 1979 Circadian Rhythms: Influences of Internal and External Factors on the Period Measured in Constant Conditions. *Zeitschrift für Tierpsychologie* **49**, 225–249.

39 Eskin, A. 1971 Some properties of the system controlling the circadian activity rhythm of sparrows. In *Biochronometry* (ed M. Menaker), pp. 55–80. Washington, DC: National Academy of Sciences.

40 Diegmann, J., Stück, A., Madeti, C. & Roenneberg, T. 2010 Entrainment elicits period aftereffects in Neurospora crassa. *Chronobiol. Int.* **27**, 1335–1347.

41 Lederbogen, F. et al. 2011 City living and urban upbringing affect neural social stress processing in humans. *Nature* **474**, 498–501. (doi:10.1038/nature10190)

42 Kantermann, T. & Roenneberg, T. 2009 Is Light-at-Night a Health Risk Factor or a Health Risk Predictor? *Chronobiol. Int.* **26**, 1069–1074.

43 Navara, K. J. & Nelson, R. J. 2007 The dark side of light at night: physiological, epidemiological, and ecological consequences. *Journal of Pineal Research* **43**, 215–224.

44 Vollmer, C., Michel, U. & Randler, C. 2012 Outdoor light at night (LAN) is correlated with eveningness in adolescents. *Chronobiol. Int.* **29**, 502–508.

45 Emerson, K. J., Bradshaw, W. E. & Holzapfel, C. M. 2008 Concordance of the circadian clock with the environment is necessary to maximize fitness in natural populations. *Evolution* **62**, 979–983.

458 **Tables**

459 **Table 1**

460 Differences between city and forest birds in start, end and duration of daily activity in the field.
461 Each response variable was standardized, respectively, on start of morning twilight, end of
462 evening twilight and daylight hours to correct for the seasonal change in daylength. Models are
463 LMMs with date, origin and τ as fixed effects. Subjects were used as random factor to correct for
464 repeated measurements.

trait	factors	estimates	s.e.m.	df	t-value	p-value
start of activity	intercept	-115.06	13.86	243	-8.30	< 0.001
	date	-0.42	0.08	243	5.94	< 0.001
	τ	9.91	5.11	9	4.10	0.0027
	origin	16.83	2.42	9	3.29	0.0093
end of activity	intercept	37.99	11.25	226	3.38	< 0.001
	date	-0.49	0.08	226	-6.35	< 0.001
	τ	1.37	5.69	9	0.24	0.8100
	origin	6.58	9.88	9	0.67	0.5200
duration of diurnal activity	intercept	87.84	27.80	220	3.16	0.0018
	date	-0.97	0.12	220	-7.80	< 0.001
	τ	1.99	5.01	9	0.40	0.6900
	origin	40.19	10.61	9	3.79	0.0043

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473 **Figure legends**

474

475 **Figure 1**

476 Differences in daily start of activity between forest and city European blackbirds in the wild.
477 Daily activity was continuously recorded on free-living forest (left, N = 6) and city (right, N = 6)
478 birds by an automated telemetry system. Start of activity was standardized to the onset of civil
479 twilight (dashed horizontal line) to correct for daily changes in photoperiod. Values indicate
480 minutes before (negative values) or after (positive values) the morning onset of civil twilight.
481 Box plots represent, from bottom to top: one standard deviation (s.d.) below the mean, lower
482 quartile, median, upper quartile and one s.d. above the mean.

483

484 **Figure 2**

485 Differences in rhythmicity index and circadian period length between forest and city blackbirds.
486 Forest (left, N = 14) and city (right, N = 14) birds were held in constant dim light of 0.3 lux and
487 their activity was continuously recorded for at least a week (10 ± 2 days). From these recordings
488 we estimated robustness of the rhythmicity (a) and period length τ (b). Since five city birds
489 showed a statistically non-significant rhythmicity index, they were excluded from the analysis of
490 period length. For box plot specification see figure 1.

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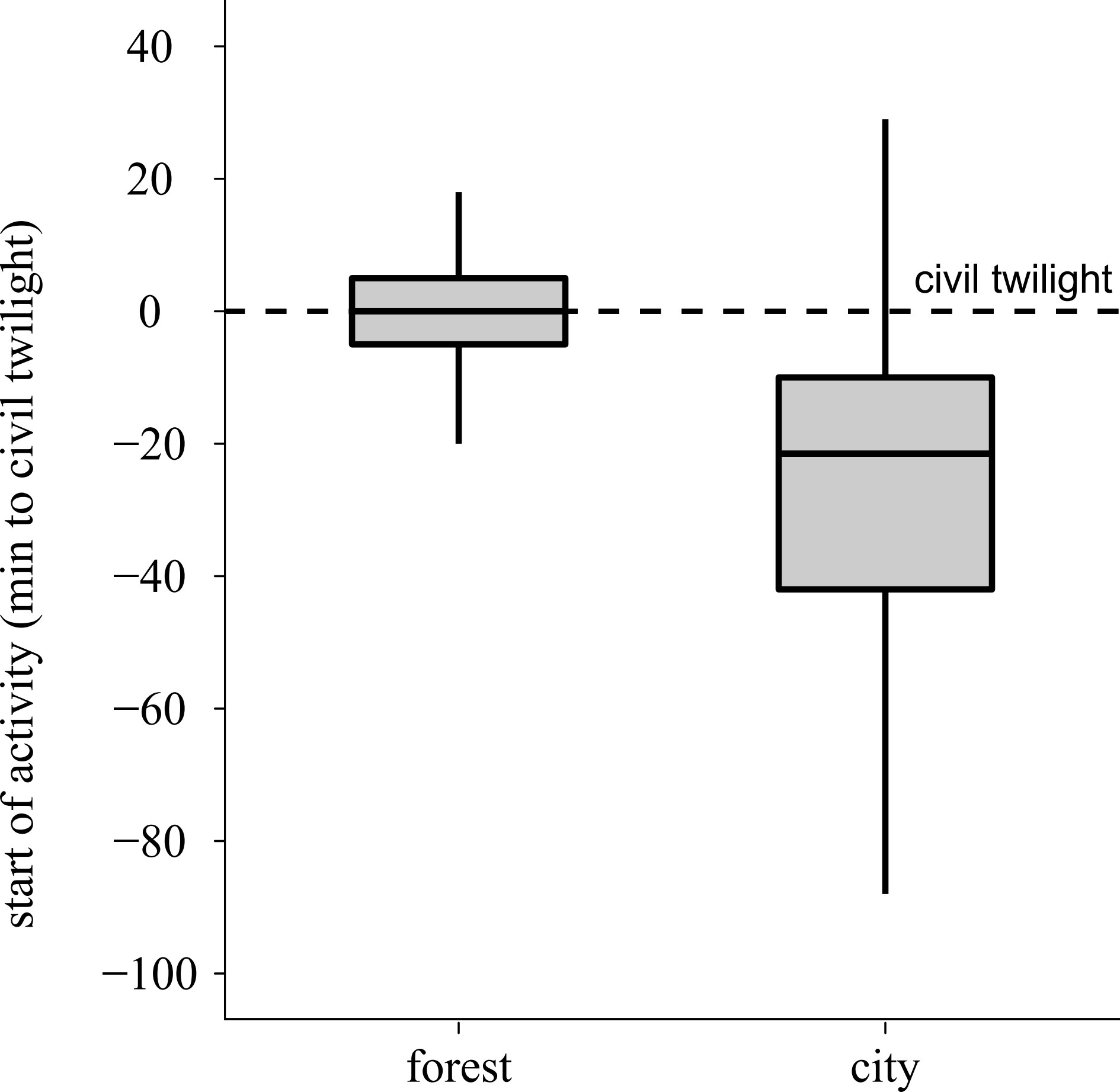
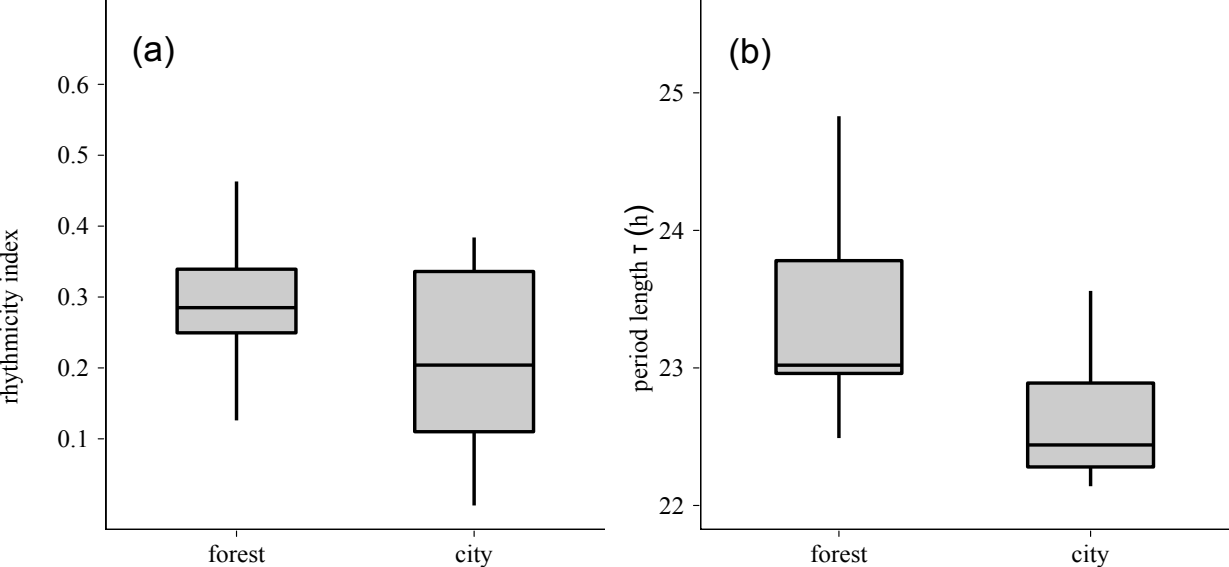
Figure 3

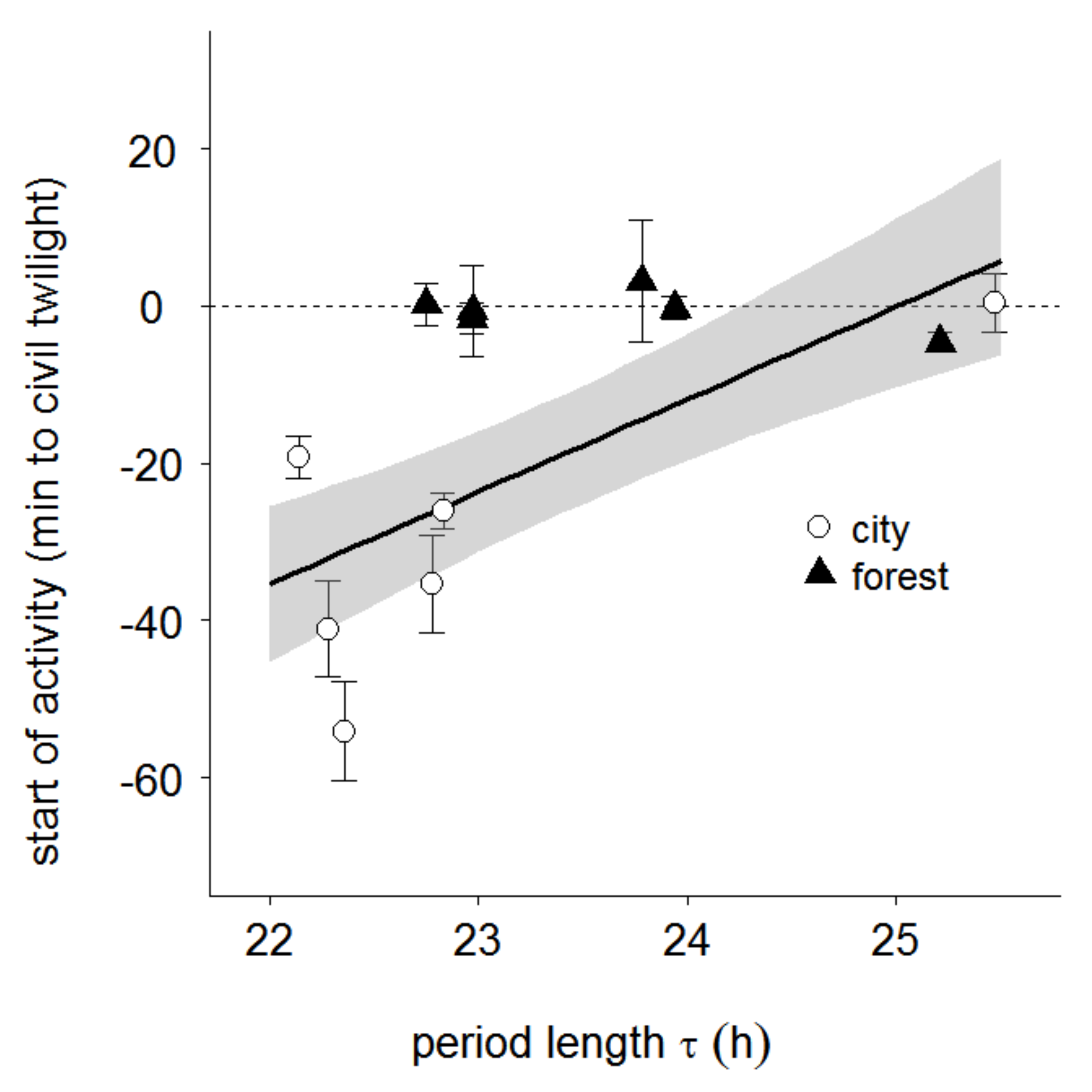
Relationship between τ and chronotype. Circadian period length predicted the time of start of morning activity in the field, relative to morning twilight. Most of the relationship is explained by city birds (white dots, N = 6), while forest birds (black triangles, N = 6) seem to be closely synchronized with daylength. Values indicate minutes before (negative values) or after (positive values) the onset of morning civil twilight (dashed horizontal line). Error bars represent mean of raw data ± s.e.m. for each individual.

Figure 4

Chronotype distribution in humans and European blackbirds in relation to *Zeitgeber* strength and city or forest environmental conditions. (a) Analysis of empirical data from human beings and application of classical oscillator theory lead Roenneberg *et al*. (2003) to postulate that chronotype distribution should depend on the *Zeitgeber* conditions a population experiences. Under strong *Zeitgebers* chronotypes should be less variable and more closely synchronized than chronotypes under weak *Zeitgebers* (*11*, graph adapted from original manuscript*)*. X-axis shows time of mid sleep point, y-axis shows relative frequency of chronotype. (b) Chronotype distributions for free-roaming city and forest blackbirds based on recordings by an automated telemetry system resemble those theoreticized by Roenneberg *et al*. City birds showed much higher variation and a lower peak in frequency of timing of start of activity than forest birds, which in turn appeared to be highly synchronized to the onset of morning twilight (dashed vertical line). X-axis shows time of start of activity, y-axis shows relative frequency of chronotype.


40
20
0
−20
−40
−60
−80
−100
start of activity (min to civil twilight)
civil twilight
forest
city


(a)
rhythmicity index
0.6
0.5
0.4
0.3
0.2
0.1
forest
city
(b)
period length τ (h)
25
24
23
22
forest
city


20
0
-20
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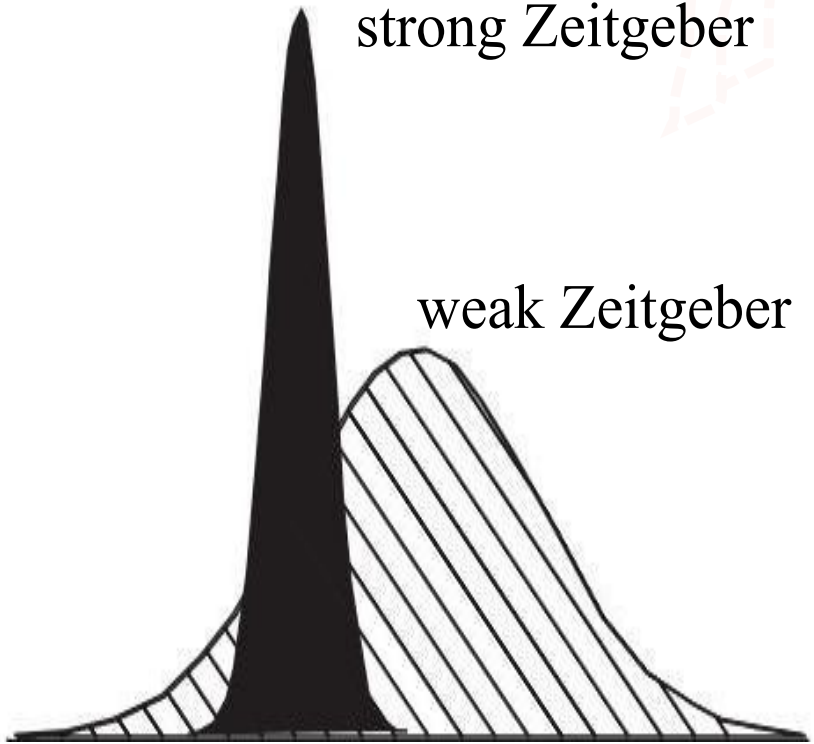
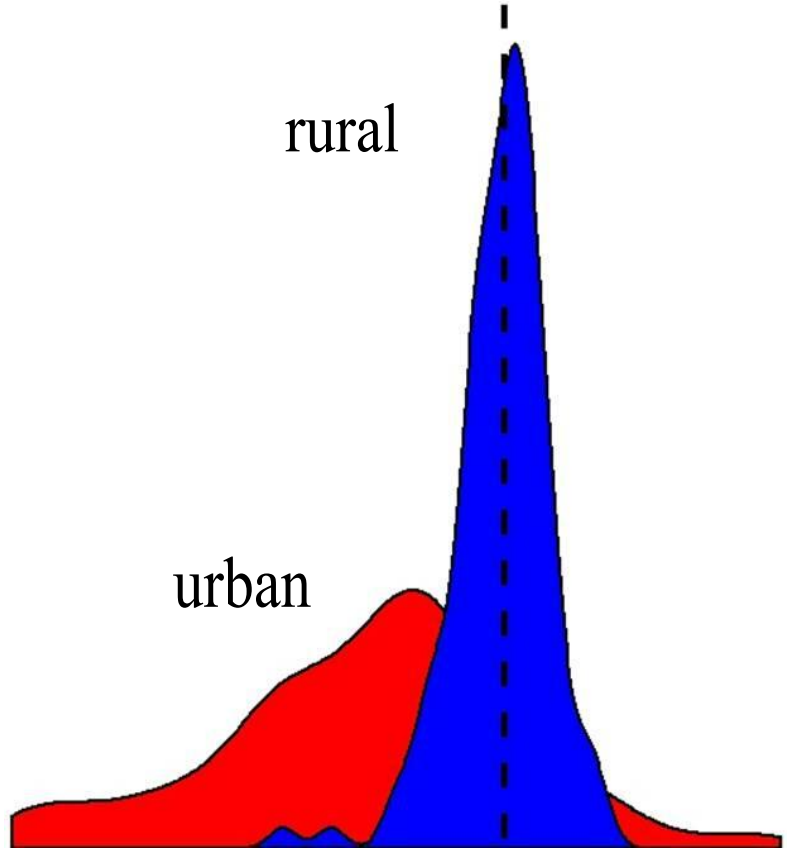
-60
start of activity (min to civil twilight)
22
23
24
25
period length τ (h)
city
forest

(a) Humans



mid sleep point

(b) Blackbirds



onset of activity

Electronic Supplementary Material

Title

Clocks for the city: circadian differences between forest and city songbirds

Author information

Dominoni, D. M, Helm, B., Lehmann, M., Dowse, H. B., Partecke, J.

Supplementary Text

(a) Entrainment to LD after LL_{dim}.

Our original experimental set-up was intended to allow us to examine how birds re-entrained to light/dark cycles after exposure to constant dim light. At the onset of the captivity experiment under LL_{dim}, birds had quickly developed rhythmic activity that started around the time predicted from the wild, and free-ran thereafter with circadian period length. This strongly indicates that under LL_{dim} we measured the circadian equivalent of regular, daytime activity. In contrast, in LD the phase and pattern of activity differed greatly from daytime activity in the field. We were surprised to see that, as evident in the examples shown in Fig. S4 below, most blackbirds failed to properly entrain to the LD cycle after release from LL_{dim}. Most birds lacked clear entrainment, and to the extent that they did entrain, they showed a predominantly paradoxical pattern of higher activity levels during the night once in LD (out of 28 birds recorded in LD after constant dim light, 7 showed irregular daily rhythms with some activity during the day and some at night (like in Fig. S4), 17 were mainly active at night, and only 4 tended to entrain to the LD cycle with activity during the light phase.). Thus, empirically, we could at best derive only poorly supported data on entrainment, and these would differ from natural entrainment to such extent that they could not be interpreted. The reasons for these patterns under LD cycles are not clear. Birds were recorded for 12 days in LD, which is usually a sufficient period of time to allow entrainment to new photoperiodic conditions [1,2]. We therefore believe that the captive conditions under LD affected the birds in a way that changed their activity patterns. A possible explanation of the prevalence of nocturnal over diurnal activity in LD could be that birds shifted into "migration/dispersal mode", which in blackbirds, like in many other songbirds, entails nocturnal migratory restlessness [3]. Migratory restlessness is in some species driven by a second oscillator with longer period length [4] and is therefore not representative of daytime activity. Mukhin et al. [5] showed that when songbirds are removed from their breeding territory and translocated away, once they are released again they switch their diel activity to becoming nocturnal while returning home. The behaviour of our birds in LD resembled this "escaping" at night strategy and might explain their entrainment to a normal light/dark cycle. Furthermore, daytime activity could be negatively masked by light in LD [6]. In all these scenarios it is highly questionable whether the activity in LD is the same activity as that in LL_{dim} and in the wild.

Supplemetary Tables

Table S1

Variance (σ^2) between and within individuals in activity traits in the field, and associated repeatability within individuals (calculated after [7]).

Onset of activity

Site	Between-ind. σ^2 (SD)	Within-ind. σ^2 (SD)	Repeatability
forest	82.35 (7.55)	80.2 (8.95)	0.51
city	352.97 (18.78)	351.04 (18.74)	0.50

End of activity

Site	Between-ind. σ^2 (SD)	Within-ind. σ^2 (SD)	Repeatability
forest	268.91 (16.40)	141.78 (11.91)	0.65
city	114.07 (10.68)	120.63 (10.98)	0.49

Duration of diurnal activity

Site	Between-ind. σ^2 (SD)	Within-ind. σ^2 (SD)	Repeatability
forest	206.22 (14.36)	198.44 (14.09)	0.51
city	163.81 (12.80)	541.92 (23.28)	0.23

Supplementary Figures

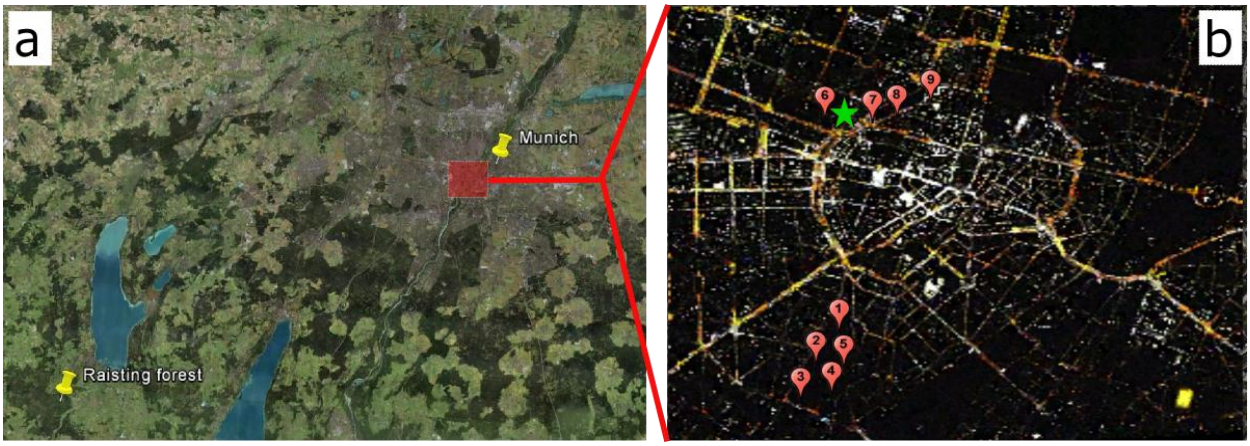


Figure S1

Study sites. Panel a: Overview map. Birds were sampled in the city of Munich and in the forest of Raisting, 40 km south-west of Munich. Panel b: Map of nocturnal light intensity in the city centre of Munich, and locations of origin of the 9 urban birds used in the analysis of circadian rhythmicity in the lab (1-5: cemetery; 6: botanical garden; 7-9: business district). Notice the higher light intensity in the business district compared to the other two locations. The green star depicts the locations where the stationary light logger (see Fig. S6) was deployed. This picture is by courtesy of Franz Kurz, DLR, Oberpfaffenhofen, Germany.

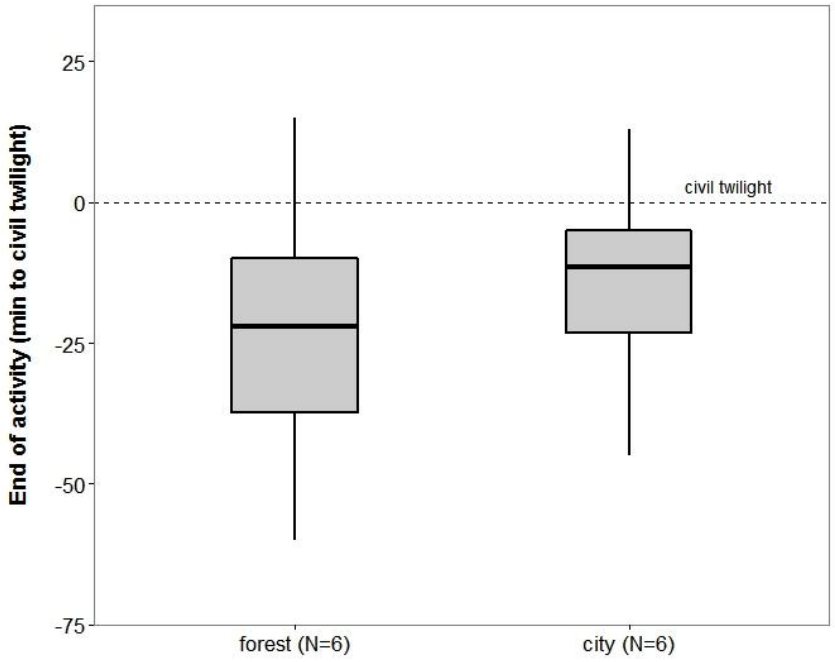


Figure S2

Difference in end of daily activity between forest and city birds. Values indicate minutes before (negative values) or after (positive values) the end of evening civil twilight (dashed horizontal line). See Fig. 1 and Methods for details on box plot specifications and statistical analysis.

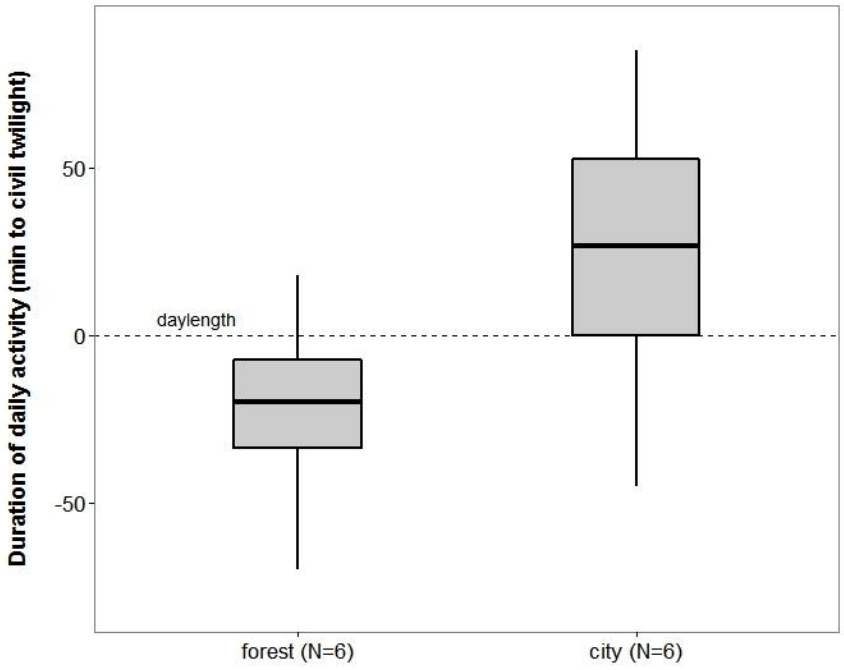


Figure S3

Difference in duration of daily activity between forest and city birds. Values indicate duration of activity relative to daylength. Negative values refer to duration of activity shorter than daylength and vice versa for positive numbers. See Fig. 1 and Methods for details on box plot specifications and statistical analysis.

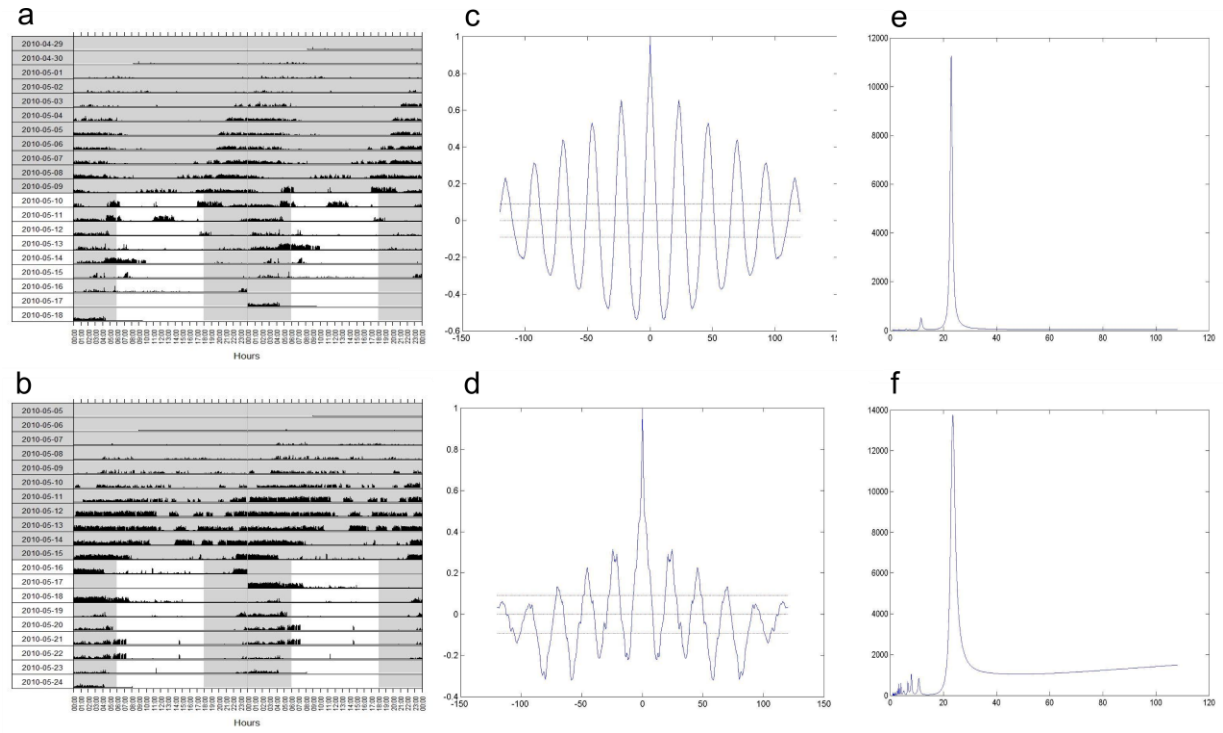


Figure S4

Spectral analysis of circadian rhythms. Two birds are taken as example, one representative of the forest (upper graph) and one of the city (lower graph) population. **a-b:** Double-plotted actograms of activity recordings in captivity. Time of the day (h) is plotted on X axis, day of experiment on Y axis. Grey shaded areas represent dim light, white areas represent simulated daylight. Only the days under LL_{dim} are used for spectral analysis. **c-d:** Autocorrelation plots. X axis shows time-lag (h), Y axis shows coefficient of correlation. The horizontal lines located above and below zero represent 95 % acceptance region for the null hypothesis of no correlation. **e-f:** Graphical representation of the estimation of circadian period length obtained after maximum entropy spectral analysis (MESA). X axis shows period length (h), Y axis shows spectral density. The period length corresponding to the highest peak indicates the main periodicity of the activity rhythm.

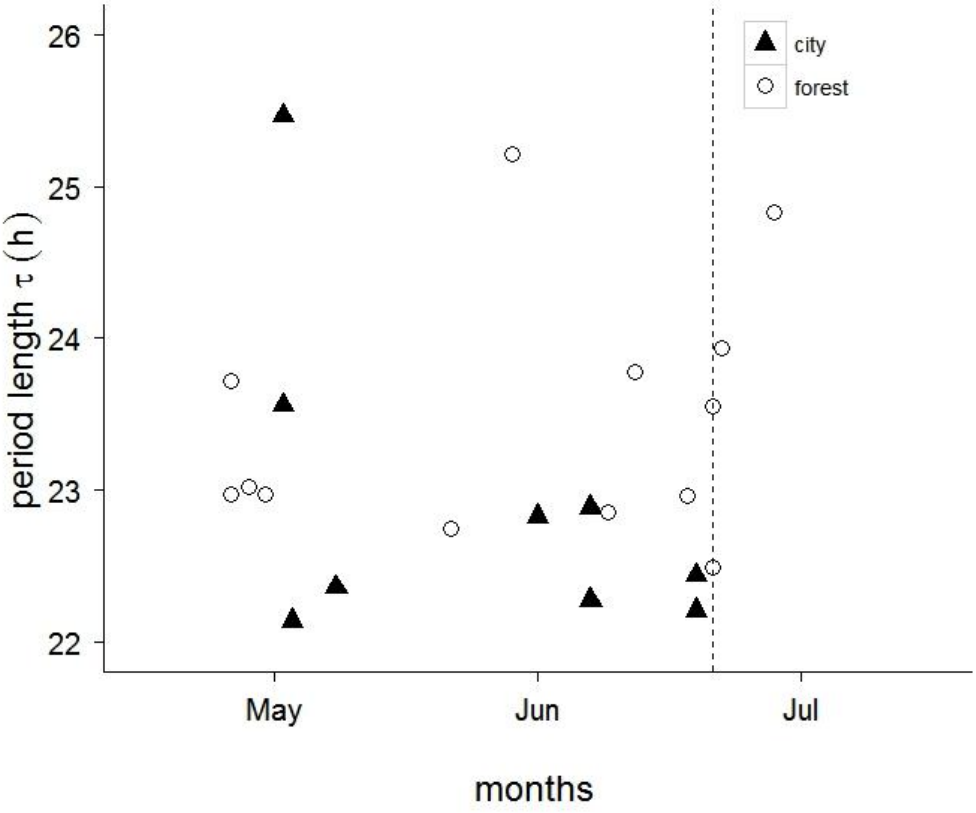


Figure S5

Relationship between period length and date. X axis represents time, Y-axis represents circadian period length τ. Rural birds are indicated by white circles and urban birds are indicated by black triangles. Vertical line notes June 21st, the day when daylength changes from increasing to decreasing daylength.

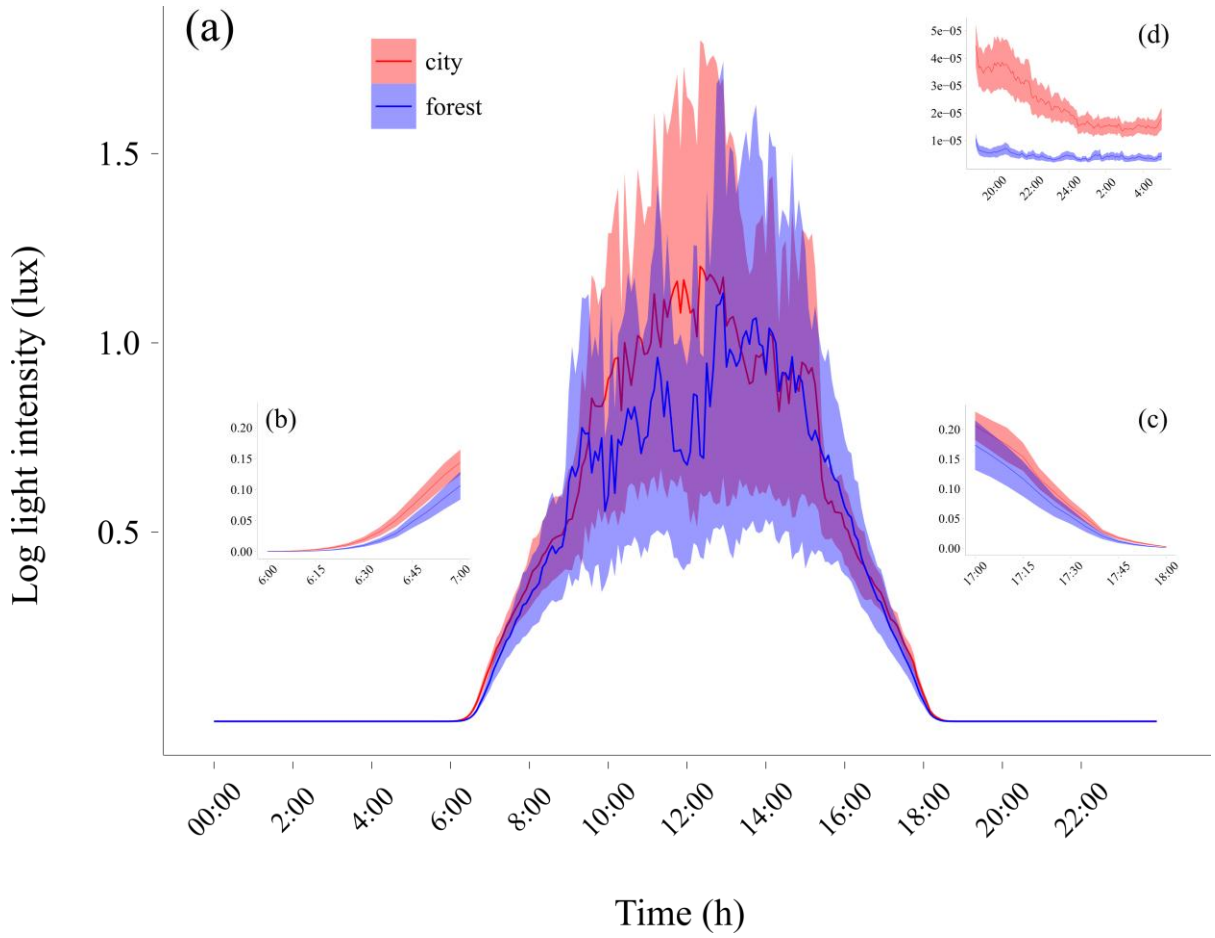


Figure S6

24-h light profiles of the urban and forest study sites. Light was recorded between March 4th and 20th, 2011, using stationary light loggers. Two loggers (Wissenschaftliche Werkstätten, University of Konstanz, Germany) were placed at a height of ~ 2 m on tree branches at the edge of a forest patch (rural site) and a botanical garden (urban site). They recorded and stored light intensity every two minutes for the entire time. We calibrated the loggers against a photometer (LI-1400 and LI-2100, LI-COR, USA) in order to convert frequency into lux values. X-axis represents time, Y axis represents log-transformed light intensity. Lines depict mean light intensities for the entire recording period, shaded areas are s.e.m. (a) Complete 24-h profile. (b) Morning twilight phase (6:00-7:00). (c) Evening twilight phase (17:00-18:00). (d) Night (20:00-4:00).

Supplementary references

1 Hau, M. & Gwinner, E. 1995 Continuous melatonin administration accelerates resynchronization following phase shifts of a light-dark cycle. *Physiology & Behavior* **58**, 89–95.

2 Abraham, U., Gwinner, E. & Hof, T. V. 2000 Exogenous melatonin reduces the resynchronization time after phase shifts of a nonphotic zeitgeber in the house sparrow (Passer domesticus). *Journal of Biological Rhythms* **15**, 48–56. (doi:10.1177/074873040001500106)

3 Partecke, J. & Gwinner, E. 2007 Increased sedentariness in European blackbirds following urbanization: A consequence of local adaptation? *Ecology* **88**, 882–890.

4 Bartell, P. A. & Gwinner, E. 2005 A separate circadian oscillator controls nocturnal migratory restlessness in the songbird Sylvia borin. *Journal of Biological Rhythms* **20**, 538–549.

5 Mukhin, A., Grinkevich, V. & Helm, B. 2009 Under cover of darkness: nocturnal life of diurnal birds. *Journal of Biological Rhythms* **24**, 225–231. (doi:10.1177/0748730409335349)

6 Aschoff, J. & Von Goetz, C. 1989 Masking of Circadian Activity Rhythms in Canaries by Light and Dark. *Journal of Biological Rhythms* **4**, 29–38. (doi:10.1177/074873048900400102)

7 Lessells, C. & Boag, P. 1987 Unrepeatable repeatabilities: a common mistake. *The Auk* **104**, 116–121.